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EFFECT OF CHITOSAN INCORPORATED WITH CUMIN AND EUCALYPTUS ESSENTIAL OILS AS ANTIMICROBIAL AGENTS ON FRESH CHICKEN MEAT

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ABSTRACT

In this study, cumin and eucalyptus essential oils were prepared and analyzed by gas chromatography–flame ionization detector. Its antibacterial effects were screened using the microdilution method for *Listeria monocytogenes*, *Salmonella typhi*, *Streptococcus pyogenes* and *Shigella dysenteriae*. The effect of the three concentrations of essential oils (0.5, 1 and 2% w/v) with 2% chitosan at 4 ± 1C temperature and storage time of up to 9 days were evaluated on the microbial quality of chicken meat. Changes in total mesophilic bacterial count (TMBC), lactic acid bacteria, Enterobacteriaceae, mold and yeast counts, and sensory properties were evaluated. The main compounds in cumin were 1,8-cineol (26.75%) and cuminaldehyde (17.1%) and the main compounds in eucalyptus were 1,8-cineol (77.32%) and limonene (8.39%). The essential oils have antibacterial effects on the four examined bacteria. In all of the treatment groups, a decrease of TMBC up to the sixth day and lactic acid bacteria, Enterobacteriaceae, mold and yeast up to the ninth day ($P < 0.05$) were observed. Overall acceptance rate in the chicken meat containing chitosan with 0.5% cumin essential oil created a better sense. Hence, the use of chitosan combined with cumin and eucalyptus might be suggested as an antibacterial packaging to extend the shelf life and as a flavor enhancer for chicken meat.

PRACTICAL APPLICATIONS

The results suggested that because chitosan film incorporated with cumin and eucalyptus can reduce meat spoilage losses and improve nutritional value, it can be used as an active packaging in the meat industry.

INTRODUCTION

Today, to maintain the quality and to extend the shelf life of meat, fish, chicken, shrimp, fruits, etc., active packaging containing essential oils is recommended. These films can reduce the risk of pathogen growth and show antioxidant activity in food systems (Appendini and Hotchkiss 2002; Contini *et al.* 2011; Samant *et al.* 2015).

Chitosan as a cationic polysaccharide is obtained by deacetylation of chitin in alkali condition. Chitosan film has an excellent potential to be used as an active compound in the packaging industry due to its biodegradable, nontoxicity, antioxidant, antifungal and antimicrobial activities as well as low permeability to oxygen (Bonilla *et al.* 2014a).

In recent years, essential oils have been used widely as natural additives in food, especially incorporated with other preservation method, which is called hurdle technology (Bonilla *et al.* 2014a; Peng and Li 2014; Calo *et al.* 2015). Therefore, these alternative preservatives can be used to replace synthetic preservatives, known as green technology (Sharafati Chaleshtori *et al.* 2011). Since these compounds possess antioxidant and antibacterial activities, they can be used in packaging materials (Tongnuanchan and Benjakul 2014). Also, essential oils, owing to their hydrophobic characteristic, can be used as barriers for water vapor, which can

improve packaging films. In essential oils, monoterpenes are highly hydrophobic. Indigenous pigments that are apart from aromatic compounds in essential oils can have an effect on color and can be used in the applications in some foods (Burt 2004; Tongnuanchan and Benjakul 2014).

Several researchers have demonstrated the antimicrobial activity of packaging incorporated with essential oils against spoilage and pathogenic microorganisms (Zivanovic *et al.* 2005; Gómez-Estaca *et al.* 2010; Bonilla *et al.* 2014b; Davidovich-Pinhas *et al.* 2014). Also, films are added with various types and amounts of essential oils to possess antioxidant activities (Moradi *et al.* 2012). In addition, films containing essential oils, due to their volatility, may have odor and flavor that cause acceptability of these films (Bonilla *et al.* 2014b; Sharafati-Chaleshtori *et al.* 2014). Therefore, the aim of this study was to evaluate the effect of chitosan incorporated with cumin (CEO) and eucalyptus essential oils (EEO) on extended storage time of fresh chicken meat during refrigerated storage.

MATERIALS AND METHODS

Preparation of the Essential Oils

The cumin and eucalyptus were gathered from the local grocery of Kashan (central of Iran) and identified by the standard botanic work in Barij Essence Co., Kashan, Iran. The CEO and EEO were prepared by steam distillation (1:5 herb/water, in w/v ratio) for 4 h using a Clevenger apparatus in Barij Essence Co. The yields of CEO and EEO were 0.4% (v/w) and 0.17% (v/w). The densities of cumin and eucalyptus at 20C were 0.879 and 0.906 g/mL.

The Gas Chromatography (GC) Analysis of Essential Oils

The GC analyses of essential oils were performed by an Agilent 6890 GC system (Waldbronn, Germany) with an HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The carrier gas (helium) was used at a flow rate of 1.0 mL/min. The GC oven temperature was kept at 40C for 1 min and programmed to 230C at a rate of 3C/min. The injector and detector temperatures were 230 and 250C, respectively. Quantitative data were obtained electronically from flame ionization detector (FID) area percent data. The main compounds of essential oils were compared with analytical standard and tested in triplicate.

Preparation of the Coating Solutions

Chitosan with medium molecular weight was purchased from Aldrich Chemical Co. (St. Louis, MO). The chitosan was dissolved with 2% (w/v) in 2% (v/v) acetic acid and stirred with a magnetic stirrer for 3 h at 55C (Fernandes *et al.* 2012). To remove the residue of insoluble particles, the chitosan solution was filtered through a Whatman No. 41 filter paper, and glycerol (Sigma Chemical Co., St. Louis, MO) was added to the chitosan solution (2% v/v) as a plasticizer. Then, CEO and EEO were added to the chitosan solution to reach final concentrations of 0.5, 1 and 2% (v/v). Tween 80 (Sigma Chemical Co.) was added to the chitosan solution to completely incorporate the essential oils.

Antibacterial Activity

The *Listeria monocytogenes* (Persian Type Culture Collection: 1163), *Salmonella typhi* (PTCC: 1609), *Streptococcus pyogenes* (PTCC: 1447) and *Shigella dysenteriae* (PTCC: 1188) were obtained from the Iranian Research Organization for Science and Technology (IROST) and cultured at 37C in tryptic soy broth (Merck, Darmstadt, Germany).

Then, for evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the standard of 0.5 McFarland was adjusted with a density of 1.5×10^8 cfu/mL in phosphate buffered saline. The MIC values for different microorganisms were demonstrated by the micro broth dilution method. The essential oils obtained were dissolved in dimethyl sulfoxide. Serial dilution method was used to show the MIC of the essential oils and chitosan film (prepared in 2% acetic acid) at concentrations of 0.175–50 mg/mL after 18 h of incubation. Each well contained 5 μL of bacterial suspension plus 95 μL of Müller-Hinton broth plus 100 μL of serial twofold dilution of the essential oil. The positive and negative control groups were 200 μL of Müller-Hinton broth and 195 μL of Müller-Hinton broth plus 5 μL of the bacterial suspensions, respectively. The MIC value, after 18 h of incubation, was identified from the first tube, without turbidity. The MBC value was recorded as the lowest concentration that showed no visible growth on Müller-Hinton agar (Merck) at 37C for 24 h (Sharafati Chaleshtori *et al.* 2013).

Chicken Collection and Preparation

Chicken breasts were purchased from a local market (Kashan, Iran) and immediately transported to the laboratory. The chicken breasts were divided into eight treatment groups as follows: (1) control sample; (2) sample coated with chitosan without essential oils; (3) sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) CEO; (4) sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) EEO. All samples were stored in refrigeration

condition (4 ± 1C), and bacteriological, chemical and sensorial tests were performed on 0, 2, 6 and 9 days.

Microbiological Analyses

Ten grams of chicken meat was diluted with 90 cm^3 of sterile saline solution (0.9%) and homogenized in a mixer (Seward Stomacher 400, Seward Medical, London, U.K.). Then, the decimal dilutions of chicken homogenates were made with saline solution and cultured on selective media for determination of total mesophilic bacterial count (TMBC), psychrotrophic bacteria, mesophilic lactic acid bacteria (MLAB), Enterobacteriaceae, yeast and mold.

About 100 μL dilutions were subsequently surface cultured twice using plate count agar (Merck). Finally, plates were incubated for 24–48 h at 37C for TMBC and 3–5 days at 20C for psychrotrophic bacteria. MLAB was cultured in de Man Rogosa and Sharpe agar (Merck) and incubated at 37C for 48. For enumeration of Enterobacteriaceae, the plates were incubated with Violet Red Bile Agar (Merck) at 37C for 24 h. Finally, yeast and mold were enumerated on yeast extract glucose chloramphenicol agar (Merck) and incubated at 30C for 3–5 days. All analyses were performed twice (Basiri *et al.* 2014).

pH Measurement

Ten grams of ground whole chicken was homogenized with 90 mL of deionized water for 1 min and was kept at room temperature for 10 min. The pH of the supernatant solution of homogenate was recorded using a pH meter (Schott pH meter, model CG824, Germany) (Basiri *et al.* 2014).

Sensory Evaluation

The overall acceptability of pan-fried-coated chicken was evaluated by a trained six-member panel. A 9-point hedonic scoring scale (dislike extremely [1], dislike very much [2], dislike moderately [3], dislike slightly [4], neither like nor dislike [5], like slightly [6], like moderately [7], like very much [8], like extremely [9]) was used for overall acceptability (Sharafati-Chaleshtori *et al.* 2014).

Statistical Analysis

The numeric data concerning the present study were expressed as means ± SD of triplicate. The significance of difference was performed by analysis of variance (ANOVA), and Duncan's multiple range test was used for mean comparison using SPSS version 16 (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered to be significant.

RESULTS

Figure 1 shows the main compounds of the essential oils. The five main compounds in EEO were 1,8-cineol (77.32%), limonene (8.39%), α-pinene (2.45%), sabinene (0.51%) and β-pinene (0.11%). The four main compounds

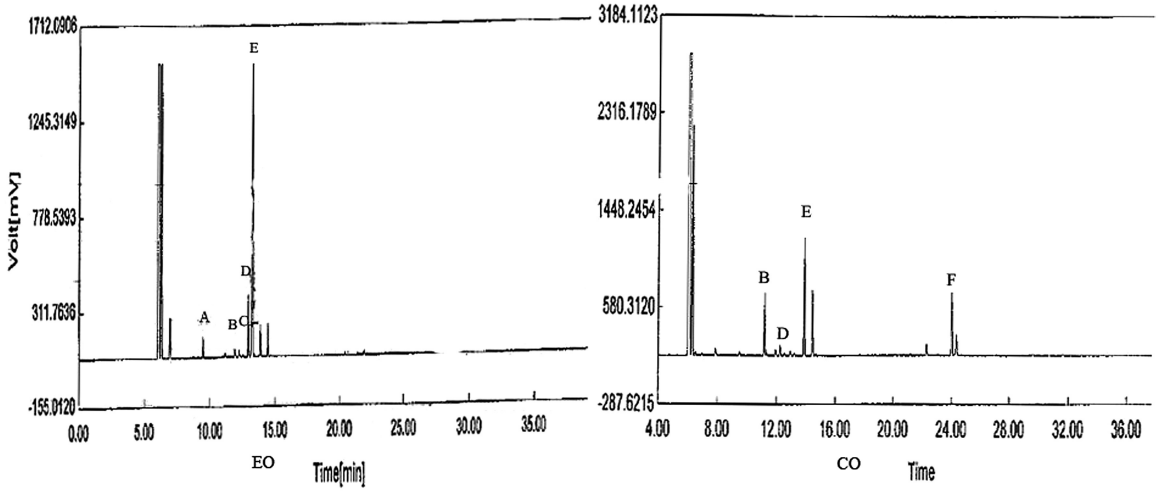

FIG. 1. MAIN CHEMICAL COMPOSITION OF ESSENTIAL OILS OF EUCALYPTUS AND CUMIN BY GAS CHROMATOGRAPHY/FLAME IONIZATION DETECTOR

EO: eucalyptus; CO: cumin; A: α-pinene (RT: 9.48); B: sabinene (RT: 11.17); C: β-pinene (RT: 11.67); D: limonene (RT: 12.95); E: 1,8-cineol (RT: 13.26); F: cuminaldehyde (RT: 23.99).

TABLE 1. MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF EUCALYPTUS, CUMIN ESSENTIAL OILS AND CHITOSAN SOLUTION (PREPARED IN 2% ACETIC ACID) ON BACTERIA

	Shigella dysenteriae		*Listeria monocytogenes*		*Salmonella typhi*		*Streptococcus pyogenes*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Eucalyptus essential oil	11.25[a]	22.5[a]	5.625[a]	11.25[a]	5.625[a]	11.25[a]	1.406[a]	2.812[a]
Cumin essential oil	0.351[b]	0.703[b]	5.625[a]	11.25[a]	5.625[a]	11.25[a]	2.812[b]	5.625[b]
Chitosan solution	0.5[c]	1[c]	0.5[b]	1[b]	1[b]	2[b]	0.25[c]	0.5[c]

Different letters a and b in the same column indicate significant differences ($P < 0.05$).

in CEO were 1,8-cineol (26.75%), cuminaldehyde (17.1%), sabinene (15.14%) and limonene (0.94%).

Table 1 shows the *in vitro* results related to the MIC and MBC. The lowest MIC value was 0.25 mg/mL for *Str. pyogenes* than chitosan solution ($P < 0.05$). Also, the most sensitive to essential oils was *Shi. dysenteriae* than CEO, with MIC value of 0.351 mg/mL ($P < 0.05$). The highest MBC value was 22.5 mg/mL for *Shi. dysenteriae* than EEO ($P < 0.05$). *L. monocytogenes* and *Salmonella typhi* have the same sensitivity to essential oils. The CEO and EEO demonstrated inhibitory effect against the above foodborne pathogenic bacteria. However, the most antibacterial activity was related to the chitosan solution.

The results in Table 2 showed that the TMBC increased in eight groups after 9 days of storage at 4 ± 1C. However, the increase in the bacterial growth was higher in the control group than in the treated groups ($P < 0.05$). Addition of chitosan coating with essential oils caused the reduction of the logarithmic growth of the bacteria up to the sixth day, but in the ninth day, the growth of bacteria increased. In the second day, the logarithmic growth in the treated groups of psychrotrophic bacteria in chicken meat decreased than the control group ($P < 0.05$), but in the sixth day, the growth of psychrotrophic bacteria increased, which was not significant between the treated groups ($P > 0.05$). In the ninth day, the most growth of the psychrotrophic bacteria was for the control group, which is equal to $1.45 \times 10^8 \pm 5 \times 10^6$ cfu/g, but the growth of the psychrotrophic bacteria for all treated groups were lower than the control group ($P < 0.05$) (Table 2). The logarithmic growth of MLAB was observed in none of the treatment groups, but in the second day, MLAB growth was observed to increase in the control group. The population of MLAB was increased to $3.45 \times 10^4 \pm 5 \times 10^2$ cfu/g after 9 days in the control group. In the ninth day, the number of MLAB in all the treated groups was significantly lower than the control group ($P < 0.05$) (Table 2). Based on the results, the control group showed an increase in the count of Enterobacteriaceae after 2 days, and on day 9, it reached $1.77 \times 10^8 \pm 2.51 \times 10^6$ cfu/g. In other treatment groups, no growth in Enterobacteriaceae was observed, which was significantly lower than the control group ($P < 0.05$) (Table 2). The results showed that there was an increase in mold and yeast counts after 2, 6 and 9 days in the control group, but those of all treated groups were less than log 2 cfu/g up to day 9. In the ninth day, the mold and yeast counts in all the treated groups were significantly lower than the control group ($P < 0.05$). The logarithmic growth of molds and yeasts was observed (Table 2).

Figure 2 shows the pH of chicken meat and chicken meat-film incorporated with CEO and EEO stored at refrigerated temperatures (4 ± 1C). Significant difference was observed between the control and treated groups in 0 day ($P < 0.05$). Based on the results, the pH level of chicken meat during storage in the control and treated group decreased at the second day. In the ninth day, the lowest pH was obtained in the chitosan with 0.5% EEO group (4.34 ± 0.00), but in the control group, it was 5.39 ± 0.01. There were significant differences between the control group and other treatment groups ($P < 0.05$). Also, the pH level of chitosan without essential oil group was higher than other treated groups ($P < 0.05$). However, no significant difference was observed between other treatment groups.

Based on the results, the use of chitosan alone as a biodegradable coating reduces the total acceptance of chicken meat so that it was lower than the control sample ($P < 0.05$). However, the lowest and highest overall acceptability were related to chitosan with 2% CEO and chitosan with 0.5% CEO ($P < 0.05$). The overall acceptability of chitosan with 1% CEO, 1% EEO and 2% EEO groups were not statistically significant ($P > 0.05$), but their overall acceptability were significantly lower than the control group and chitosan with 0.5% CEO ($P < 0.05$). The use of essential oils caused a significant increase in the overall acceptance of the samples ($P < 0.05$). The results can be seen in Fig. 3.

DISCUSSION

In a previous work, the essential oil of *Cuminum cyminum* contained α-pinene (29.2%), limonene (21.7%), 1,8-cineol (18.1%) and linalool (10.5%) (Mohammadpour *et al.* 2012). In the present study, the major components of CEO were 1,8-cineol (26.75%), cuminaldehyde (17.1%), sabinene (15.14%) and limonene (0.94%). Elaissi *et al.* (2012) demonstrated that the major constituents of the eight

TABLE 2. THE MICROBIOLOGICAL STATUS OF CHICKEN MEAT AND CHICKEN MEAT-FILM WITH DIFFERENT CONCENTRATIONS OF CUMIN AND EUCALYPTUS ESSENTIAL OILS OF STORED AT REFRIGERATED TEMPERATURES (4 ± 1C) (CFU/G)

		Days			
Groups		0	2	6	9
Total mesophilic bacterial count	C	$4.9 \times 10^4 \pm 10^{3a}$	$2.83 \times 10^5 \pm 10^{3a}$	$2.83 \times 10^8 \pm 5.77 \times 10^{3a}$	$3.1 \times 10^9 \pm 10^{8a}$
	Ch	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$2.68 \times 10^4 \pm 7.63 \times 10^{2b}$
	CZ 0.5	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$2.23 \times 10^5 \pm 1.52 \times 10^{3b}$
	CZ 1	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$2.16 \times 10^5 \pm 1.51 \times 10^{3b}$
	CZ 2	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$2.09 \times 10^5 \pm 1.53 \times 10^{3b}$
	CE 0.5	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$1.99 \times 10^5 \pm 10^{3b}$
	CE 1	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$4.06 \times 10^4 \pm 5.77 \times 10^{2b}$
	CE 2	$4.9 \times 10^4 \pm 10^{3a}$	$<10^{2b}$	$<10^{2b}$	$3.1 \times 10^4 \pm 10^{3b}$
Psychrotrophic bacteria	C	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$2.66 \times 10^5 \pm 5.77 \times 10^{3a}$	$8.1 \times 10^7 \pm 10^{6a}$	$1.45 \times 10^8 \pm 5 \times 10^{6a}$
	Ch	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$1.47 \times 10^4 \pm 2.51 \times 10^{2b}$	$1.48 \times 10^4 \pm 6.35 \times 10^{2b}$
	CZ 0.5	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$2.93 \times 10^5 \pm 2.08 \times 10^{3b}$
	CZ 1	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$2.85 \times 10^2 \pm 5 \times 10^{b}$	$2.74 \times 10^5 \pm 2.08 \times 10^{3b}$
	CZ 2	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$2.68 \times 10^5 \pm 5.77 \times 10^{2b}$
	CE 0.5	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$2.89 \times 10^5 \pm 10^{3b}$
	CE 1	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$2.63 \times 10^2 \pm 3 \times 10^{b}$	$2.85 \times 10^5 \pm 10^{3b}$
	CE 2	$3.3 \times 10^3 \pm 2.08 \times 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$2.59 \times 10^5 \pm 10^{3b}$
Mesophilic lactic acid bacteria	C	$<10^{2a}$	$2.5 \times 10^4 \pm 10^{3a}$	$2.83 \times 10^7 \pm 5.77 \times 10^{5a}$	$3.45 \times 10^4 \pm 5 \times 10^{2a}$
	Ch	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 0.5	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 1	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 2	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 0.5	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 1	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 2	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
Enterobacteriaceae	C	$<10^{2a}$	$3.16 \times 10^3 \pm 5.7 \times 10^{a}$	$3.1 \times 10^7 \pm 10^{6a}$	$1.77 \times 10^8 \pm 2.51 \times 10^{6a}$
	Ch	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 0.5	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 1	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 2	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 0.5	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 1	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 2	$<10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
Yeast and mold	C	$3.1 \times 10^3 \pm 10^{2a}$	$2.13 \times 10^5 \pm 5.77 \times 10^{3a}$	$2.56 \times 10^7 \pm 5.77 \times 10^{5a}$	$7.83 \times 10^7 \pm 2.08 \times 10^{6a}$
	Ch	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 0.5	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 1	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CZ 2	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 0.5	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 1	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$
	CE 2	$3.1 \times 10^3 \pm 10^{2a}$	$<10^{2b}$	$<10^{2b}$	$<10^{2b}$

C: control; Ch: chitosan; CZ 0.5, 1 and 2: sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) cumin essential oil; CE 0.5, 1 and 2: sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) eucalyptus essential oil. Different letters a and b in the same column indicate significant differences ($P < 0.05$).

eucalyptus species included 1,8-cineol (42–70.2%), α-pinene (1–22%), limonene (0.4–4.4%). In the present work, EEO contained 1,8-cineol (77.32%), limonene (8.39%) and α-pinene (2.45%).

Various compounds in essential oils are related to factors such as geographic location, plant growth phase, temperature, the land factor and harvesting time of the plant, genetic and environmental factors (Goze *et al.* 2009). The phenolic compounds present in essential oils can inhibit oxidation, giving a hydrogen atom to free radicals and prevents injury to cells, and show antioxidant properties (Deba *et al.* 2008; Erel *et al.* 2012; Sharafati-Chaleshtori *et al.* 2014).

In the present work, antibacterial effects of CEO and EEO were observed *in vitro* (Table 1). Previous studies have demonstrated antibacterial, antifungal and antiviral properties

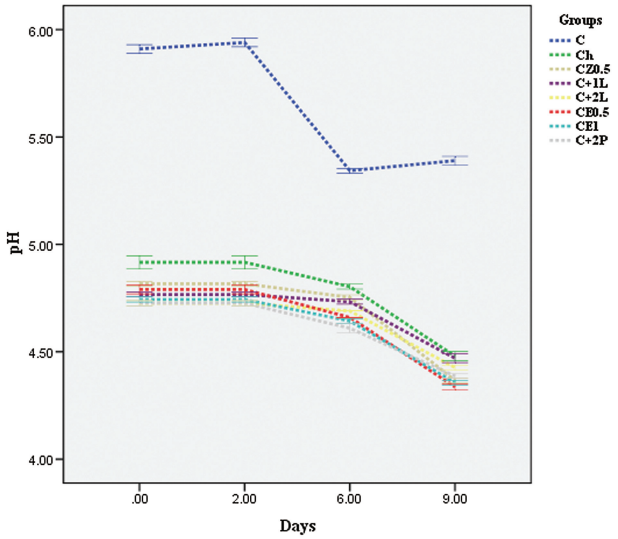

FIG. 2. pH OF CHICKEN MEAT AND CHICKEN MEAT FILM STORED AT REFRIGERATED TEMPERATURES (4 ± 1C)
C: control; Ch: chitosan; CZ 0.5, C + 1L and C + 2L: sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) cumin essential oil; CE 0.5, CE1 and C + 2P: sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) eucalyptus essential oil.

of limonene, eugenol, linalool and spathulenol in essential oils (Elaissi *et al.* 2012; Mohammadpour *et al.* 2012). Lis-Balchin and Deans (1977) demonstrated the antilisterial activity of essential oils containing large amounts of 1,8-cineole. Also, Van Vuuren and Viljoen (2007) showed that combination of 1,8-cineole and limonene has synergistic antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Tserennadmid *et al.* (2011) reported that α-pinene as a monoterpene hydrocarbon incorporated with limonene or linalool has additive and synergistic effects against *Saccharomyces cerevisiae*. The monoterpenes in essential oils are able to destroy the cellular respiratory and transmission of ions to bacteria, demonstrating antibacterial activity (Deba *et al.* 2008; Sharafati Chaleshtori *et al.* 2015). Several studies have reported antimicrobial and antifungal activity of CEO and EEO against gram-negative and gram-positive bacteria, as well as mold such as *Aspergillus* (Hajlaoui *et al.* 2010; Tyagi and Malik 2011; Elaissi *et al.* 2012; Mohammadpour *et al.* 2012). However, the mechanism of the antimicrobial activities of essential oils remains unclear (Mohammadpour *et al.* 2012). With regard to the above discussion, phenolic compounds, particularly monoterpenes, might be the predominant compound for this effect, although other compounds should be involved, too. There are a lot of medicinal plants possessing these compounds (Shirzad *et al.* 2011; Rabiei *et al.* 2014). Hence, these plants might also have antimicrobial activities that are worth examining.

With regard to new viewpoints to replace chemical preservatives with natural origin and unwanted effects, the essential oils as natural preservatives, several studies have evaluated the antioxidant and antibacterial activities of various essential oils in food models (Viuda-Martos *et al.* 2010; Wu *et al.* 2014; Samant *et al.* 2015). Nowadays, in food

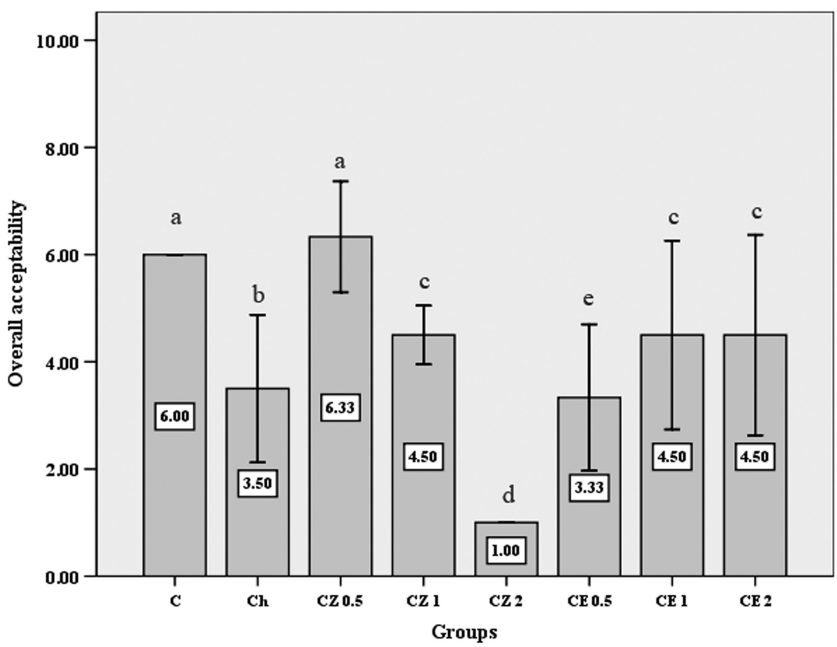

FIG. 3. OVERALL ACCEPTABILITY EVALUATION OF COOKED CHICKEN MEAT AND CHICKEN MEAT FILM INCORPORATED WITH DIFFERENT CONCENTRATIONS OF CUMIN AND EUCALYPTUS ESSENTIAL OILS
C: control; Ch: chitosan; CZ 0.5, 1 and 2: sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) cumin essential oil; CE 0.5, 1 and 2: sample coated with chitosan incorporated with 0.5, 1 and 2% (v/v) eucalyptus essential oil. Different letters (a, b, c, d) in the same column indicate significant differences among the different films for the overall acceptability. Data reported ($n = 6$) are mean values.

hygiene, hurdle technology is used to extend the shelf life of perishable foods (Sharafati-Chaleshtori *et al*. 2014; Wu *et al*. 2014).

The results of this study showed that in the treatment group, the total mesophilic bacterial count (TMBC) during storage in the refrigerated condition increased in all groups (Table 2). Addition of chitosan coating with CEO and EEO caused the reduction of the logarithmic growth of the bacteria up to sixth day, but in the ninth day, the growth of bacteria increased. The logarithmic growth of psychrotrophic bacteria showed a decrease during the second day in the treatment groups, but this growth in the ninth day increased, which was significantly less than that of the control group ($P < 0.05$). The logarithmic growth of MLAB was observed in none of the treatment groups, but in the second day, an increase of MLAB growth in the control group was observed (Table 2). No increase in the number of Enterobacteriaceae count was observed in the treated groups, but the control group showed an increase in the count of Enterobacteriaceae after 2 days and on the ninth day. In all the treated groups, the amounts of mold and yeast were less than log 2 cfu/g up to day 9, but in the control group, an increase in mold and yeast counts in the second, sixth and ninth day was observed (Table 2). Chitosan causes the death of bacteria due to its positively charged amine groups and reaction to anionic groups of bacterial cell surface (Wu *et al*. 2013). Chitosan film incorporated with CEO and EEO increased antibacterial effects. The presence of phenolic compounds in essential oils can increase the permeability and can remove cytoplasmic contents by attacking phospholipids of the cell membrane. Also, it can affect the enzymes in the cellular walls of the bacteria (Wu *et al*. 2013, 2014).

Previous studies have demonstrated the antibacterial effects of gelatin compounds with chitosan and oregano essential oil (Wu *et al*. 2013) and carboxymethyl cellulose–polyvinyl alcohol films with clove oil in ground chicken meat. Also, the efficacy of these films was shown against *Bacillus cereus* and *Sta. aureus* in ground chicken meat (Muppalla *et al*. 2014). In a previous study, the coating effect of food prepared by apple mixed with antibacterial compounds in the prevention of growth of *L. monocytogenes* and *Salmonella enteritidis* in chicken meat was examined and it was proposed that it could be used to prevent contamination of nutritive materials because of its antibacterial compounds (Ravishankar *et al*. 2009). Sánchez-Ortega *et al*. (2014) reported that antibacterial compounds such as essential oils, extracts and antibiotics such as nicin could be gradually released into food when these compounds are added to food alone.

Based on the results, the pH level of chicken meat decreased during the storage period in the control and treated groups up to 12 days (Fig. 2). There were significant differences between the control group and other treatment groups ($P < 0.05$). Also, the pH level of chitosan without essential oil group was higher than other treated groups ($P < 0.05$). However, no significant difference was observed between other treatment groups. Initial decrease in pH levels can be due to the use of chitosan coating prepared in 2% acetic acid, but with increased storage time at the temperature of 4 ± 1C, the pH level of chicken meat decreased, which might be caused by microorganism growth (Basiri *et al*. 2014). In another study of treated chicken meats containing *Zataria multiflora* and *Mentha longifolia* essential oils, pH values were lower than that of the control group during storage time (Behnam and Aliakbarlou 2014). The results of this study are in accordance with the results of other researchers who have demonstrated the effects of coatings on reducing the pH value in different products (Basiri *et al*. 2014; Raeisi *et al*. 2014; Wu *et al*. 2014).

Based on the results shown in Fig. 3, the use of chitosan alone as a biodegradable coating reduces the total acceptance of food lower than the control sample. However, the lowest overall acceptability was related to chitosan with 2% CEO. While the use of essential oils causes an increase in the overall acceptability of the samples, but in this study, all of the treated groups except chitosan with 0.5% CEO had lower overall acceptability than the control group. In previous studies, the addition of essential oils and extracts increased the acceptability of a variety of foods (Busatta and Et 2008; Amany *et al*. 2010). Petrou *et al*. (2012) demonstrated that the addition of chitosan combined with 0.25% oregano essential oil increased the acceptability of chicken breast meat. It is worth mentioning that considering the taste of chitosan and its preparation by acidic methods, essential oils and extracts can be used as harmless compounds to increase the taste and odor, but increase in the concentration of essential oils and extracts to the amount that does not trigger organoleptic adverse effects is justified; however, in the case of occurrence of this effect, it is not recommended for use in high concentrations.

Antioxidants generally scavenge the free radicals and reduce the food degradation; however, in specific conditions and in high concentrations, they may act as pro-oxidant and help in food degradation (Rafieian-kopaei *et al*. 2014). Hence, in high concentrations, pro-oxidant activities of essential oils seem to be the cause of reduction in their qualities.

The toxicity of some of these compounds in *in vivo* situations might also be due to their pro-oxidant activities (Taghikhani *et al*. 2013). However, in practice the pro-oxidant activity can be beneficial because by imposing a mild degree of oxidative stress, the levels of biotransformation enzymes and antioxidant defenses might increase, protecting the cells more efficiently (Bahmani *et al*. 2014). However, the taste and the acceptability of the food may

decrease. It should be noted that the extent to which these compounds might act as antioxidant or pro-oxidant is still poorly understood and requires further studies.

CONCLUSION

With attention to antibacterial activities of CEO and EEO, antimicrobial and biodegradable chitosan film can be wildly used in the food packaging and preservation industries.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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